Synopsis

Title: Deciphering the Mechanisms of AMPK Activation upon Anchorage-Deprivation

AMP-activated protein kinase (AMPK) is a key regulator of energy homeostasis in cells. It has been implicated as a therapeutic target for various metabolic diseases like type II diabetes and obesity. However, its role in cancer is context-dependent and therefore warrants further studies to explore its possible use as a therapeutic target. AMPK can either promote or retard the growth of cancer cells depending on other cues and stresses in the milieu of the cancer cells. This study aims to understand AMPK signaling in response to extracellular cues of matrix deprivation and matrix stiffness that are important determinants of metastasis.

1) Calcium-Oxidant Signaling Network Regulates AMPK Activation upon Matrix Deprivation.

Recent work from our lab, as well as others, has identified a novel role for the cellular energy sensor AMP-activated protein kinase in epithelial cancer cell survival under matrix deprivation. However, the molecular mechanisms that activate AMPK upon matrix-detachment remain unexplored. In this study, we show that AMPK activation is a rapid and sustained phenomenon upon matrix deprivation, while re-attachment to the matrix leads to its dephosphorylation and inactivation. Since matrix-detachment leads to loss of integrin signaling, we investigate whether integrin signaling negatively regulates AMPK activation. However, modulation of FAK or Src, the major downstream components of integrin signaling, fails to cause a corresponding change in AMPK signaling. Further investigations reveal that the upstream AMPK kinases, LKB1 and CaMKKβ, contribute to AMPK activation upon detachment. Additionally, we show LKB1 phosphorylation and cytosolic translocation upon matrix deprivation, which might also contribute to AMPK activation. In LKB1-deficient cells, we find AMPK activation to be predominantly dependent on CaMKKβ. We observe no change in ATP levels under detached conditions at early time points suggesting that rapid AMPK activation upon detachment is not triggered by energy stress. We demonstrate that matrix deprivation leads to a spike in intracellular calcium as well as oxidant signaling and both these

intracellular messengers contribute to rapid AMPK activation upon detachment. We further show that ER calcium release induced store-operated calcium entry (SOCE) contributes to intracellular calcium increase, leading to ROS production, and AMPK activation. We additionally show that the LKB1/CaMKK-AMPK axis and intracellular calcium levels play a critical role in anchorage-independent cancer sphere formation. We find a significant increase in LKB1 as well as pACC levels in breast tumour tissues in comparison to normal tissues. Further, we observe a significant correlation between LKB1 and pACC levels in breast tumour tissues suggesting that LKB1-AMPK signaling pathway is active in vivo in breast cancers. Thus, the Ca^{2+}/ROS triggered LKB1/CaMKK-AMPK signaling cascade may provide a quick, adaptable switch to promote survival of metastasising cancer cells.

2) Extracellular Matrix Stiffness Regulates Stemness through AMPK.

Cancer cells experience changes in extracellular matrix stiffness during cancer progression. However, the signaling pathways utilised in sensing matrix stiffness are poorly understood. In this study, we identify AMPK pathway as a possible mechanosensory pathway in response to matrix stiffness. AMPK activity, as measured by downstream target phosphorylation, is found to be higher in soft matrix conditions. We additionally show that compared to stiff matrices, soft matrices increase stemness properties, as evidenced by the increased expression of stemness markers, which is dependent on AMPK activity. Thus, we elucidate a novel mechanotransduction pathway triggered by matrix stiffness that contributes to stemness of cancer cells by regulating AMPK activity.

Taken together, our study identifies a novel calcium-oxidant signaling network in the rapid modulation of AMPK signaling in the context of matrix detachment. This pathway is especially relevant in the context of metastasising cancer cells that may not face energy stress in the blood stream but are matrix-deprived. Inhibition of AMPK might compromise the viability of these circulating cells thereby reducing the metastatic spread of cancer. Our study further suggests that varying stiffnesses experienced by cancer cells can modulate AMPK activity and this, in turn, regulates stem-like properties. Thus our study provides novel insights into various extracellular cues that regulate this kinase and

contribute to cell survival and metastasis. This knowledge can be utilised in the stage-specific use of AMPK inhibitors in the treatment of breast cancer patients.